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Beluga whales in James Bay: a separate entity from eastern Hudson Bay belugas?

Le béluga dans la baie James : une entité distincte de la population de l'est de la baie d'Hudson ?

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ABSTRACT

Satellite telemetry and genetic analyses of mitochondrial DNA sequences and nuclear DNA microsatellites were used to determine if the beluga whales found in James Bay remain in the bay for the winter and if these whales comprise a distinct summer stock and breeding population. Over three years of tagging (2007-2009), 12 tagged whales showed no movement out of James Bay during the winter. Population genetic analyses of these whales, along with other samples from James Bay and compared to adjacent locations in western Hudson Bay, eastern Hudson Bay and the Belcher Islands, confirmed that belugas in James Bay form a distinct stock from other management stocks in Hudson Bay. However, the beluga whales in James Bay were weakly differentiated (possibly due to a high degree of admixture) from whales in all the other locations and they were significantly more related to each other than belugas in other locations. These results suggest the presence of a local breeding population that has recently diverged, mixes to some extent with groups of whales in other areas, and/or are being hunted at the edge of their range by hunters from Sanikiluaq and east Hudson Bay. Based on the combination of all the information from this study, James Bay should be considered a separate stock for surveys, population estimates and management of the EHB stock.

RÉSUMÉ

Nous avons utilisé des techniques de télémétrie par satellite et des analyses génétiques de séquences d'ADN mitochondrial et de microsatellites d'ADN nucléaire pour déterminer si les bélugas observés dans la baie James y demeurent pendant l'hiver et si ces animaux constituent un stock estival distinct et une population reproductrice isolée. Aucun des 12 bélugas équipés d'émetteurs satellites au cours des années 2007–2009 n'a effectué de mouvement en dehors de la baie James durant l'hiver. Les analyses génétiques des échantillons prélevés sur ces individus, ainsi que sur d'autres bélugas de la baie et des régions adjacentes (parties est et ouest de la baie d'Hudson, îles Belcher) confirment que les bélugas de la baie James forment un stock estival distinct des autres stocks de la baie d'Hudson. Cependant, ces bélugas étaient faiblement différenciés des animaux des autres régions (peut-être à cause d'un degrés élevé de mélange) et davantage apparentés les uns aux autres. Ces résultats suggèrent la présence d'une population reproductrice locale qui aurait divergé récemment, se mêle dans une certaine mesure aux groupes des régions voisines, ou qui est chassée à la limite de son aire de distribution par les chasseurs de Sanikiluaq et de l'est de la baie d'Hudson. L'ensemble des informations apportées par cette étude permet de conclure que les bélugas de la baie James devraient être considérés comme un stock distinct pour les relevés, les estimés d'abondance et à fins de gestion du stock de l'est de la baie d'Hudson.

INTRODUCTION

Beluga whales are widely distributed throughout coastal Hudson Bay. Based on observations of reoccurring aggregations in particular estuaries, Reeves and Mitchell (1987) outlined a management framework where the summering aggregations formed separate management stocks. Since then, photo-identification (Caron and Smith 1990), genetic and contaminant studies (Brennin et al. 1997, Brown Gladden et al. 1999, de March et al. 2004) have provided evidence that individual beluga return every year to the same aggregation areas. Moreover, telemetry studies in Nunavut (Richard et al. 2001, Richard and Stewart 2009) and northern Quebec (Nunavik) (Lewis et al. 2009) have shown that tracked individuals from specific summering aggregations within the summer season did not overlap in distribution. This cumulative evidence provides additional support for the concept of discrete summer stocks (Smith and Hammill 1986) and has led to the current use of summering stocks as management units (e.g., Richard 2010).

Nunavik communities have traditionally harvested beluga belonging mostly to populations: the Western Hudson Bay population (WHB), which numbers about 57,000 individuals (Richard 2005), and the Eastern Hudson Bay population (EHB), which was depleted by intensive commercial hunting between the 1860's and the early 1900's and has decreased from an estimated pristine population size of 12,500 to about 3,100 individuals in 2010 (Doniol-Valcroze et al. 2011). Both populations undertake seasonal migrations through Hudson Strait, to their winter grounds in Hudson Strait and the Labrador Sea.

Belugas have been observed in James Bay in winter (Jonkell 1969) but are seen by locals mainly in summer when they approach the coastline and enter river mouths (Sergeant and Brodie 1975, Richard 2010). The relationship of these whales to the WHB and EHB animals, to whales moving around the Belcher Islands (Sanikiluaq), and their patterns of seasonal movement remain unclear. They were initially considered part of the EHB population and were believed to move between the Eastmain River in James Bay and the Nastapoka, Little Whale and Great Whale river estuaries in eastern Hudson Bay (Finley 1982). Dedicated summer aerial surveys in James Bay began in 1985 (Smith and Hammill 1986) and since then, separate population estimates have been provided for this summer aggregation (Kingsley 2000, Gosselin et al. 2002, 2009).

Belugas in James Bay are of particular interest for management purposes because of their geographic proximity to EHB whales and their relatively large population size (~9,000, Gosselin et al. 2009). Here, we summarize the most recent results of genetic analyses and satellite telemetry to clarify the relationship between James Bay beluga and neighbouring stocks.

METHODS

GENETIC IDENTITY

Samples and data for genetic analyses

Beluga samples have been collected for many years as part of harvest sampling programs, satellite tagging studies, biopsy programs, and other biological sampling efforts. Samples in the field were preserved in a salt-saturated 20% DMSO solution (Seutin et al. 1991) and frozen upon arrival at the lab. Total cellular DNA extractions were performed using a variety of methods including phenol:choloform (Amos and Holzel 1991), Qiagen spin columns (DNeasy Blood and Tissue kits), and the Biosprint automated platform (Qiagen Inc, Valencia, CA, USA).

In most cases, molecular determination of sex was completed using methods described in Bérubé and Palsbøll (1996) or Shaw et al. (2005).

Portions of these samples have been analyzed for a number of previous genetic studies (Mancuso 1995; Brennin et al. 1997; Brown Gladden et al. 1999; Murray et al. 1998) using varying amounts of genetic information and a variety of statistical approaches. These studies have been summarized in de March and Maiers (2001) and expanded on in the same document and in de March and Postma (2003). Recently, Turgeon et al. (2009) pooled data collected from several of these studies and added new additional genetic data to create a more complete and validated dataset to conduct a genetic mixture analysis of Hudson Bay beluga.

For this study, a subset of the data from Turgeon et al. (2009) was combined with additional samples from James Bay and Sanikiluaq that had not previously been analyzed (Table 1). Approximately 40 (7%) samples from the Turgeon dataset were re-analyzed for microsatellite information to correct for discrepancies in allele scoring between labs and to estimate error rate in the data. Samples that were missing too much information (3 or more loci) and samples that were identified as being potential duplicate samples were removed from the dataset leaving n=461 (for haplotype distribution analyses) or n=433 samples (for haplotype diversity and F_{st} analyses) for mitochondrial DNA (mtDNA) sequence analyses and n=580 samples for microsatellite analyses.

Mitochondrial DNA sequencing

Mitochondrial DNA haplotypes were generated using 609bp of sequence in the mtDNA control region. Primers Belmt-5 (GAT AGA GTT TTT TGA GCC CG) and Belmt-6 (TCA CCA CCA ACA CCC AAA G) were used in a polymerase chain reaction (PCR) mixture containing 1x buffer, 25mM MgCl2, 10mM dNTP mix, 20uM of each primer, 0.5units of Taq polymerase and approximately 50-200ng of template DNA. The PCR temperature profile was as follows: 95C for 10min.; 35 cycles of 95C for 20s, 55C for 30s, 72C for 45s; extension at 72C for10 min. Products were visualized using agarose gel electrophoresis and successfully amplified samples were cleaned using QiaQuick PCR clean-up kits (Qiagen Inc.). DNA sequencing was performed using BigDye ver3.5 (Applied Biosystems) with the Belmt-6 primer as the sequencing primer (2uM). The PCR temperature profile was: 96C for 1min.; 32 cycles of 96C for 10s, 50C for 30s, and 60C for 4min; and an extension at 72C for 7min. Sequencing was performed on an Applied Biosystems 3130xl genetic analyzer (Life Technologies).

DNA sequences were aligned and edited using MEGA ver 5 (Tamura et al. 2011) and haplotypes identified using GenAlEx 6 (Peakall and Smouse 2006). The resulting haplotypes, designated "E-types", are different than those used in earlier studies of Hudson Bay beluga genetics and from the haplotypes ("H-types") used for genetic mixture analysis in Turgeon et al. (2009). Turgeon et al. (2009) opted to use a shorter region of sequence that allowed for a more complete dataset yielding better sample sizes for the type of analysis that was being applied to the data. In this paper, the purpose of the analyses was to determine if James Bay and east Hudson Bay beluga comprise different stocks. In 2003, de March et al. presented a working paper to the DFO National Marine Mammal Peer Review Committee evaluating the usefulness of analyzing extended or modified mtDNA sequences for stock discrimination of Hudson Bay beluga (unpubl. data). The conclusion was that haplotypes based on a greater number of variable positions were able to divide ubiquitous "H-type" haplotypes into new "E-type" haplotypes that were common in different geographic locations. This appeared to assist in defining stocks and populations.

Table 1. Beluga sample locations and samples sizes by year. Numbers highlighted in grey are newly analyzed data. All other samples are data from Turgeon et al. (2009 and 2012).

Area	Location	Year	N samples
West Hudson Bay	Churchill	1989	7
		1990	12
		1991	7
		1992	13
		1993	14
		2002	1
	Nelson River	2002-04	11
		Total	**65**
James Bay	Cape Jones Island	2003-04	5
	James Bay	2002	5
		2003	2
		2004	2
	James Bay	**2007**	**6**
		2008	**6**
		2009	**5**
	Northend Long Island	2003	2
		2004	8
		2005	**4**
		2010	**3**
	Middle Long island	**2005**	**1**
		2007	**3**
		2008	**3**
		2009	**2**
	Southend Long Island	2003	4
		Total	**61**
East Hudson Bay	Inukjuak	1994	7
		1997	10
		1998	13
		1999	16
		2000	18
		2001	14
	King George Islands	**2005**	**5**
	Nastapoka River	1984	18
		1985	24
		1999-2000	9
	Umiujaq	1994-2002	12
	Little Whale River	2003	13
	Kuujjuarapik	1990-98	32
		2000-01	13
		2002-04	9
		Total	**213**
Belcher Islands	Sanikiluaq	1993	10
		1994	30
		1995	23
		1996	19
		1997	19
		1998	3

2002	10
2003	14
2004spring	20
2004summer	10
2004entrap	8
2005	16
2006	5
2007	12
2008	16
2009	18
2010	13
2011harvest	6
2011entrap	7
Total	**259**

Nuclear DNA microsatellites

After data were merged from both laboratories, 10 microsatellite loci were available to generate genetic profiles using primers from several sources with the associated reaction conditions (EV94 from Valsecci and Amos 1996; FCB1, 3, 4, 5, 8, 10, 11, 14, 17 from Buchanan et al. 1996). Loci were amplified individually or in duplexes in reactions with a total volume of 10uL. Amplification products were analyzed using an Applied Biosystems 3130xl genetic analyzer (Life Technologies) with an internal 400HD Rox size standard. Alleles were scored according to size in base pairs using GeneMarker ver1.95 software (SoftGenetics).

Genetic data analyses

Mitochondrial DNA:

In the past, proportions of beluga harvests in Hudson Strait and from Sanikiluaq have been identified as "EHB-type" or "not-EHB-type" for management purposes on the basis of variable mtDNA sequence positions and frequency of occurrence in different locations. This identification system has been continued and updated for the purposes of this paper. This will then be used to identify the frequency of "EHB-type" belugas sampled in James Bay as is the practice for whales harvested along Hudson Strait and in the Belcher Islands.

The most suitable model of evolution to use when calculating sequence diversity statistics and constructing phylogenetic trees was determined using the model selection module in MEGA 5 (Tamura et al. 2011). This analysis determined the Tamura (1992) model to be most appropriate and was implemented where appropriate. Genetic diversity and differentiation was assessed using Arlequin ver3.5 (Excoffier et al. 2005). Genetic diversity was quantified as gene diversity (probability that two randomly chosen haplotypes will be the same) and as nucleotide diversity (probability that two randomly chosen nucleotides at the same position are identical). Differentiation among groups was calculated using haplotype frequencies (Weir and Cockerham 1984) and significance was evaluated using permutations. AMOVA was used to determine how the variation in the dataset was partitioned.

Initial mtDNA sequence alignments and haplotype identifications were checked in the program DNA Alignment ver1.3.1.1 (Fluxus Technology). This program was then used to prepare data to illustrate relationships among haplotypes as a median-joining network using the program Network ver4.6.0.0 (Fluxus Technology). Given the number of haplotypes in this dataset, the initial network was quite complex. The network was simplified somewhat by applying a star contraction algorithm described in Forster et al. (2001). This algorithm allows for the production

of a "skeleton" network where clusters of related sequences that form star-like topography are collapsed into a single ancestral node. A specific mutational distance radius (defined by the user) is used to identify clusters. This value is used in the algorithm to identify and combine similar sequences into a common ancestral node (details in Forster et al. 2001). This series of steps may be repeated to progressively contract the network.

Nuclear DNA:
The entire dataset was examined for matching samples using GenAlEx ver6.41 (Peakall and Smouse 2006). Loci were evaluated for deviations from Hardy-Weinberg expectations and for linkage disequilibrium using FSTAT ver2.9.3.2 (Goudet, 1995) both globally and within the four groupings (i.e. East Hudson Bay, West Hudson Bay, James Bay and Sanikiluaq). Bonferonni corrections were applied to correct for multiple tests (Rice 1989). The following measures were calculated using FSTAT ver2.9.3.2 (Goudet, 1995) to evaluate the genetic variability within groups: observed and expected heterozygosity; number of alleles and allelic richness; and inbreeding coefficients; GenAlEx ver6.41 (Peakall and Smouse 2006) was used to calculate relatedness with the Queller and Goodnight (1989) estimator.

Population structure was investigated using the program STRUCTURE ver2.2 (Pritchard et al. 2000), which searches for clusters of samples in the data that conform to population expectations (HWE and LD) using a Bayesian approach. The beluga data set was analyzed in STRUCTURE using the correlated alleles model (Falush et al. 2003) and with the admixture parameter (alpha) inferred. The analysis was initiated with a 500,000 cycle burn-in followed by 2,000,000 MCMC repetitions. Three independent runs were preformed for each modeled number of clusters (K) and K was evaluated from one to ten.

Calculation of frequency based measure of differentiation among the four groups (F_{ST}) was conducted using FSTAT ver2.9.3.2 (Goudet, 1995). An Inter-class (between-class) eigenanalysis (inter-class PCA) was used to visualize genetic differentiation among three of the four groups, which were expected to not be mixed stock samples (EHB, JB, and WHB). Inter-class PCA maximizes the variance among groups, whereas PCA maximizes the total variance (Jombart 2008). Individuals and groups are visualized along the axis that maximally separates groups. Inter-class PCA provides an ad-hoc assessment of how well groups are partitioning the variation. These analyses were performed in the statistical package R (www.r-project.org), using the package *adagent* (Jombart 2008) to manage the genetic data and *ade4* (Chessel et al. 2008) to conduct the PCA. The Sanikiluaq samples were not used in this analysis, thus the groups for comparison were James Bay, Western Hudson Bay and Eastern Hudson Bay. Beluga harvested in Sanikiluaq and the Belcher Islands have the potential to be a unique summer stock but may also be from a mixed stock that has variable proportions of other stocks depending on the seasonal timing of the hunt (de March and Postma 2003, Turgeon et al. 2009). This mixed stock signal requires further dedicated analysis, therefore these samples were left out for the objectives of this paper to simplify the visualization of the relationship of James Bay whales to East Hudson Bay whales.

SATELLITE TELEMETRY

Deployment of satellite transmitters

From 2007 to 2009, 14 belugas (11 females and 3 males) were captured at Cape Hope Island (James Bay) using shore anchored nets, and equipped with TD-SRDLs (Temperature-Depth Satellite Relayed Data Loggers) secured to the dorsal ridge (Kingsley et al. 2001). Of these 14 beluga whales, 12 individuals had records that extended beyond the summer season and were kept for analysis of large-scale movements (Table 2).

Table 2. Summary of satellite transmitter deployment on beluga whales in James Bay. Individuals #44452 and ##44483 were not kept in the analysis of long-term movements.

ID	Year	Sex	Deployment date	Last transmission
44436	2007	F	Aug 08	Oct 30
44485	2007	M	Aug 20	Nov 12
44436	2008	F	Aug 16	Jan 12
44446	2008	F	Aug 06	Nov 29
44447	2008	F	Aug 08	Dec 23
44451	2008	F	Aug 08	Dec 1
44452	2008	F	Aug 10	Sep 26
44477	2008	F	Aug 16	Mar 13
44478	2009	M	Aug 09	Dec 4
44479	2009	F	Jul 30	Jan 2
44480	2009	F	Jul 31	Jan 23
44481	2009	F	Aug 10	Jan 13
44482	2009	F	Aug 02	Feb 11
44483	2009	F	Aug 01	Sep 28

Data treatment and mapping

Data from satellite transmitters were processed automatically at the Sea Mammal Research Unit (SMRU), St. Andrews, UK, and were made available in a standardized database format. Animal locations were obtained via the ARGOS satellite system. Accuracy varied depending on transmission classes: 150 m, 350 m, 1 km, and >1 km for classes 3 through 0, respectively. Classes A, B, and Z had undetermined accuracies.

Proportion of migrants in population

To estimate the proportion of non-migrants in the population from the satellite telemetry results, we classified each individual into migrant and non-migrant categories based on whether ARGOS locations showed movement outside of James Bay at any time during deployment (i.e., north of 55° N). We modeled the number of migrants with a binomial distribution: $X \sim \text{Bin}(N, p)$, where X is the number of migrants amongst N, the number of beluga, and p is the true proportion of migrants in the population. Based on x the number of migrants in the sample and n the number of beluga equipped with satellite transmitters known from the data, we first estimated p with the unbiased estimator $\overline{p} = x/n$, which is also the maximum likelihood estimator.

There are several ways to derive a confidence interval for $\overline{p}$ but few of them are recommended when n is small and p is close to 0. In such cases, it is common to use a Bayesian approach for inference on p (Berger 1985). Beta distributions are the standard conjugate priors for binomial distributions. If p has a prior distribution Beta(α_1, α_2), then its posterior distribution is Beta($X + \alpha_1$, $N - X + \alpha_2$). We used the Jeffreys interval, which can be regarded as a continuity-corrected version of the commonly used Clopper-Pearson interval (Brown et al. 2001). The non-informative Jeffreys prior is Beta (0.5, 0.5). Thus, the Jeffreys interval is a $100(1 - \alpha)\%$ Bayesian interval which endpoints are the $\alpha/2$ and $1-\alpha/2$ quantiles of the Beta($x + 0.5$, $n - x + 0.5$) distribution. The exception is for $x = 0$ where the lower limit is modified to avoid the undesirable result that the coverage probability tends to zero as $p \rightarrow 0$.

We use the corresponding cumulative distribution function to estimate the median value of $\overline{p}$, which has no closed form but can be derived numerically.

RESULTS

GENETIC IDENTITY

mtDNA sequencing

Mitochondrial DNA sequences were obtained for 433 samples and categorized into 45 haplotypes. Gene and nucleotide diversity was lowest in James Bay and Western Hudson Bay however these were also the stocks with the lowest sample sizes (60 samples each; Table 3). Variation in the data set (using AMOVA - analysis of molecular variance) was partitioned such that 16% was among groups while 83% was within groups (P <0.000). The level of differentiation among groups was moderate and significant in all pairwise comparisons (Table 4).

Table 3. Gene and nucleotide diversity for mitochondrial DNA sequences among 45 haplotypes found in beluga samples used in this study. Standard deviation (SD) for the gene diversity is determined for the sampling process, SD for nucleotide diversity is determined for both the sampling and stochastic processes.

Stock (or Group)	N	N Haplotypes	Gene diversity	Nucleotide diversity
Eastern Hudson Bay	127	18	0.862 +/- 0.018	0.0082 +/- 0.0044
Western Hudson Bay	60	13	0.771 +/- 0.036	0.0028 +/- 0.0019
James Bay	60	11	0.742 +/- 0.049	0.0064 +/- 0.0036
Sanikiluaq	186	31	0.876 +/- 0.016	0.0063 +/- 0.0035

Table 4. F_{ST} values (indicating degree of genetic differentiation) for a pairwise population comparison using mtDNA sequences of beluga samples from main areas for this study.

FST	Eastern Hudson Bay	James Bay	Western Hudson Bay	Sanikiluaq
Eastern Hudson Bay	0	***	***	***
James Bay	0.19346	0	***	***
Western Hudson Bay	0.18037	0.24322	0	***
Sanikiluaq	0.13106	0.18387	0.1713	0

Comparison of E-type haplotype sequences using only variable positions reveals that the haplotypes that have been previously identified as "EHB-type" haplotypes (B. de March and L. Postma unpubl. data) are very distinct (Figure 1). All of these haplotypes share a common motif that is five or more mutations different from all other haplotypes. This can be visualized using a median-joining network (Figure 2) that clearly distinguishes the "EHB-type" haplotypes from the "not-EHB" haplotypes. In addition, some previous haplotypes (e.g., E41, E81, E32) and new haplotypes (e.g., E116 and others) have been resolved for classification. Sequence information for St. Lawrence River beluga samples (L. Postma unpubl, data) were included as an external reference, however the main haplotypes from these samples (E16, E160 and E161) were clearly grouped with the "EHB-type" haplotypes.

Variable Position (in 609bp total sequence)

```
                  2234445889111111111222222222333333334445
                  2440249456001266677112466888004444691 98
                      476505734780814245013456925 58
                                  Haplotype:
Ref     TTCCTTTGTTGCTCACACCCTCCTTTTCTACTAACATAC
E01     .......A.....................................
E02     ............................C.......
E03     ...........................C..........
E04     .......................C......C.......
E05     ............................C.....G.
E06     ............................C......T
E07     ............................CG.....T
E08     .......................C.....CG.....T
E09     ................T.........C..........
E10     ..............G..............C.......
E11     .......A.........T...........TC.......
E12     .......A......G...T...........TC.....G.
E13     ....C..AC.A...G.....C..........C.......
E15     ....C..AC.A...G.....C.....C....C.G.....
E16     ....C..AC.A..TG.....C.....C....C.....G.
E17     ....C..AC.A..TG.....C.....C..G.......G.
E18     ....C..AC.A..TG.....C.....C..G.C.....G.
E19     ....C..AC.A..TG.....C....CC..G.C.....G.
E22     ..................C..........C.......
E23     ..........T...................C.......
E24     ......................T........C.......
E25     ..........A..................................
E28     ................T.....................
E29     ..............G.................C.......
E30     ..........T..................................
E31     ....C..AC.A..TG....TC.....C....C.......
E32     ....C..AC.A..T......C....CC....C.......
E33     ................T.........G.........
E34     ........................C.............
E35     ..................T...........C.......
E36     ......................T..............
E37     ......................T.....C..........
E38     .......AC.........T...........TC.......
E40     ........................C.....C.......
E41     ....C..AC.A..TG.....C.....C....C.......
E42     ............T.....................
E50     ..............G..............................
E51     ....C..AC.A..TG.....C..........C.....G.
E52     ................................C.....GT
E55     .........C.....................C.......
E56     ....C..AC.A..TG.....C.....C....C.G.....
E57     ......CA..........T...........TC.......
E58     ........................C.....CG....G.
E59     ................G.....................
E61     .......A...T......T...........TC.......
E62     .......A.........T...........T...T.....
E63     ..T....AC.........T..........TC.......
E65     ............................C..C.......
E67     ..........T......T...........C.......
E68     ..................T.....................
E69     ....C..AC.A..TG.....C....CC....C.......
E70     ..........T........C..........C.......
E71     ................................G.........
E72     ..............................................
E73     ..................T...T........C.......
E74     C.............................TC.......
E75     .......AC.........T..........TC.......
E76     .......A.........TT..........TC.......
E77     ..............G.G..............C.....G.
E79     ..............G.G..............CG....G.
E80     ..................T...........TC.......
```

Figure 1. Mitochondrial DNA sequence positions defining beluga haplotypes using a reference sequence sample that is haplotype E72 ("not-EHB-type"). Haplotypes highlighted in yellow correspond to haplotypes designated as "EHB-types", in blue are haplotypes that are found in the majority of St. Lawrence River beluga samples and in grey is a haplotype found in both EHB and St. Lawrence River. All other haplotypes are considered "not-EHB-types".

Variable Position (in 609bp total sequence)

```
223444588911111111122222222233333333445
244024945600126667711246688800444469198
476505734780814245013456925 58
```

Haplotype:

```
E81   ....C..AC.A..TGT....C.....C....C.......
E82   ....C..AC.A..TG.....C.....C..G.C.......
E83   ..............................C.G.....
E84   ....C..AC.A...G.....C.....C....C.G...G.
E86   ................T..G........TC.......
E88   .......A..........T..........GTC.......
E90   ..............G.............TC.......
E92   .....C.......................C.......
E94   ....C..AC.A..TG.G...C.....C...TC.....G.
E95   .......A........G.T...........TC.......
E96   .......A..A.......T...........TC.......
E97   .......A..........T......C....TC.......
E100  .C............G.G.............C.....G.
E101  ............................T...........
E103  .......A..........T...........TC....C..
E108  ....C..AC.A..TG..T.TC.....C....C.......
E109  .......A...T.TG...T...........TC.......
E110  .......A.........TT...........TC.......
E113  ...............G..............C.....G.
E114  ....C..AC.A..TG.....C.....C....C...G.G.
E115  ..........A.....................C.......
E116  .......A..........T...T....T..TC.......
E117  ....C..AC.A.CTG.....C.....C....C.G.....
E119  ....C..AC.A..TG.....C.T...C....C.G.....
E120  .......A..........T...........TC.....G.
E121  .......A........G.............TC.....G.
E123  ...................C.........G.C.......
E124  ................GT.....................
E127  ...................C.....................
E129  ....C..AC.A..TG..T..C.....C....C.G.....
E130  ................................CG......
E131  ..................T.............C.....G.
E132  ....C..AC.A..TG.....C.....C..........G.
E133  ..................................G.....
E138  ....................................G.
E140  .......A.......................TC.....G.
E141  ..............................T........
E142  .......AC.........T....C.......TC.......
E144  .......A.....T....T...........TC.....G.
E146  .......AC.........TT...........TC.....G.
E147  .......AC.........T...........TC.....G.
E148  .......A..........T...T.......TC.....G.
E149  ....C..A..A..TG.....C.....C....C.....G.
E150  .......A........G.T...........TC.....G.
E153  ....C..AC.A...G...T.C.....C....C.G.....
E157  .......A..........T.........C.TC.......
E160  ....C..A..A..TG..TT.C.....C....C.....G.
E161  ....C..AC.A..TG..TT.C.....C....C.....G.
E162  ...T...A..........T...........TC.......
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Figure 1(con't). Mitochondrial DNA sequence positions defining beluga haplotypes. Haplotypes highlighted in yellow correspond to haplotypes designated as "EHB-types", in blue are haplotypes that are found in the majority of St. Lawrence River beluga samples and in grey is a haplotype found in both EHB and St. Lawrence River. All other haplotypes are considered "not-EHB-types".

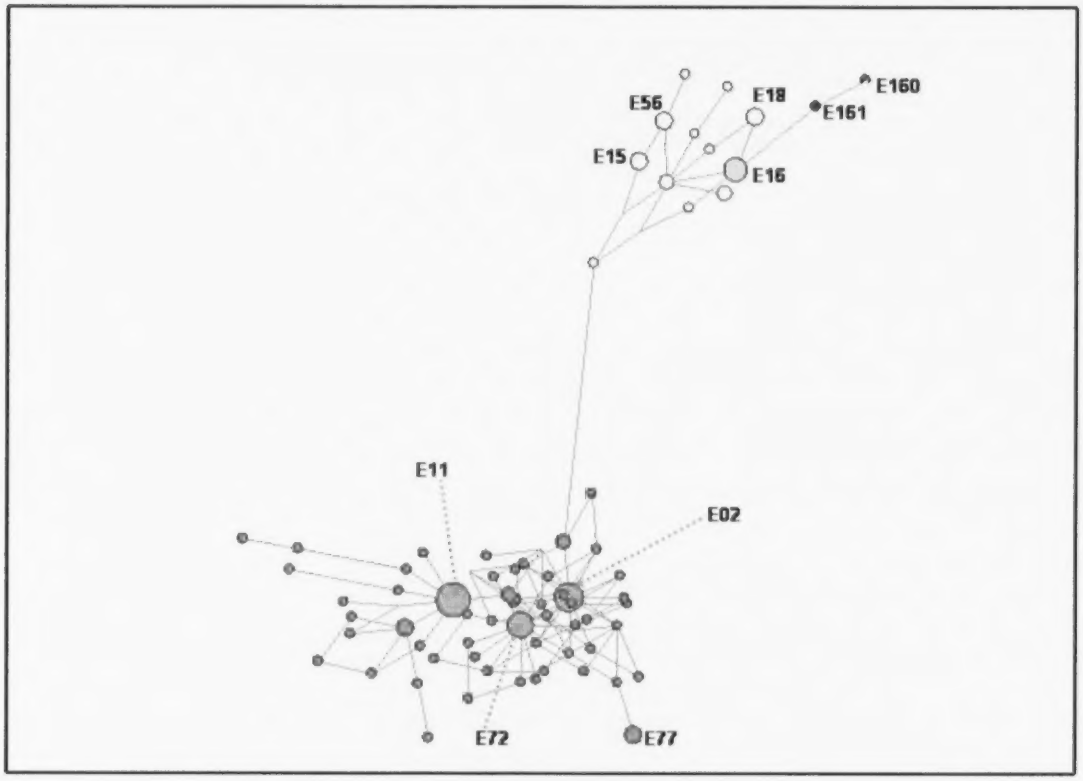

Figure 2. Median joining network illustrating the relationships of mtDNA haplotypes based on the number of mutations between individual haplotype sequences (i.e. mutations at variable sequence positions – see Figure 1). A star contraction algorithm to simplify the network (see Methods section of text) and collapsed haplotypes are represented by larger circles, with the size of the circle representing the number of haplotypes making up the node. Main haplotypes of interest are labeled.

The distribution of haplotypes was characterized by a common haplotype found in all areas (E02), those shared among a subset of areas (e.g., E11, E72) and uncommon haplotypes found only in one area (e.g., E116 in James Bay, E06 and E10 in Sanikiluaq, E16 in EHB) (Figure 3). F_{ST} analysis of haplotype frequencies found that sample groups from each of the four areas were significantly differentiated from each other (Table 4). However, the proportion of "EHB-type" haplotypes in the samples from James Bay was 9/60 (15%). Of these haplotypes, 4/9 were E32 which is classified by the network as an "EHB-type" haplotype, but is not present in the samples from the EHB arc used in this study. These "EHB-type" haplotypes were all from samples collected around Long Island, found near the coast at the northeast tip of James Bay where it opens into Hudson Bay. The samples from Cape Jones Island (south of Long Island into the northwest corner of James Bay) were a mixture of E11 (n=2), E116 (n=2) and E57 (n=1). The samples analyzed from the belugas tagged at Cape Hope Island, in the middle section of James Bay, were entirely composed of E11 (n=17).

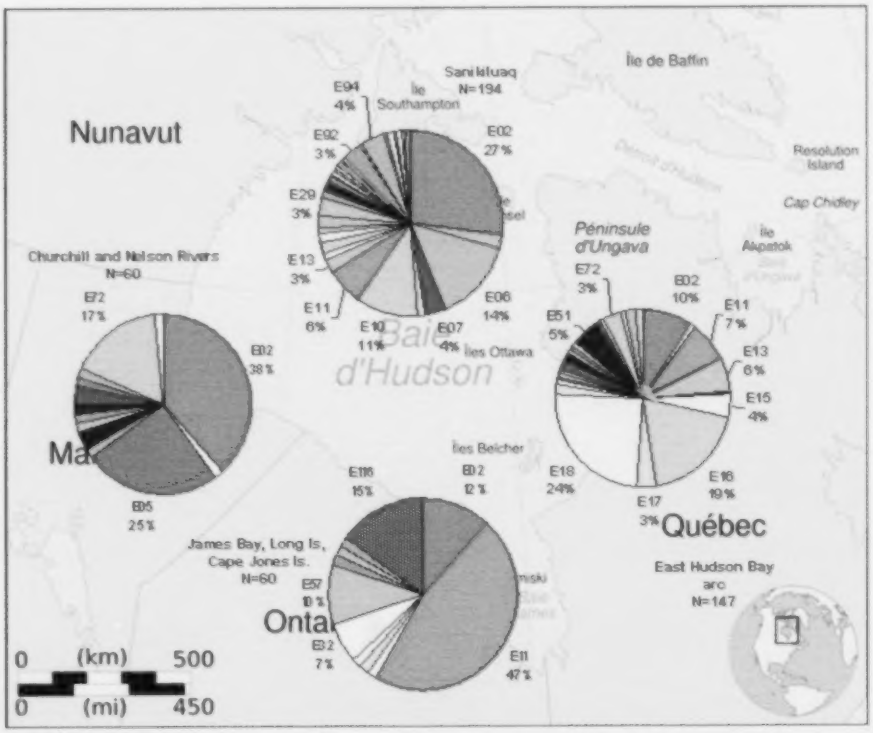

Figure 3. Distribution of haplotypes in four beluga Hudson Bay summering areas. Haplotypes of frequencies greater than 2% are labelled to highlight similarities and differences among areas.

Nuclear DNA microsatellites

Microsatellite profiles at ten loci were obtained for 580 unique samples. No deviations from HWE were detected and no two loci were linked. Measures of genetic variability can be found in Table 5. James Bay samples are significantly more related to one another compared to the null expectation (relatedness is equal to zero = individuals are unrelated) and compared to the other groups; they also are slightly less variable (Figure 4). Pairwise F_{ST} values indicate that there is significant differentiation among all groups except between EHB and WHB (Table 6). Comparisons of differentiation between James Bay and other groups are an order of magnitude larger than other significant comparisons. The STRUCTURE analysis was less informative. Applying Pritchard (Pritchard et al. 2000) criteria indicated that all samples analyzed are from one single cluster (K=1; Figure 5). However, the criteria of Evanno (Evanno et al. 2005) suggested that two clusters existed (Figure 6), though this method in fact cannot suggest that a single cluster exists. At K=2 or K=3, there is some support (Figure 6) that James Bay beluga may be more strongly associated with one cluster. Nevertheless, several individuals in James Bay, as well as other areas, are strongly and almost equally admixed which undermines evidence of distinct clusters. If clusters do exist, extensive gene flow probably occurs.

Table 5. Summary of gene and nucleotide diversity measure at microsatellite loci for samples groups by area.

Sample group	Sample size	Unbiased Hz	Unbiased Hz SD	Obs Hz	Obs Hz SD	No Alleles	No Alleles SD	Rarified Allelic Richness (50)	R	F_{IS}
Eastern Hudson Bay	202	0.7221	0.0378	0.7260	0.0100	8.80	3.79	7.11	0.003	-0.005
Western Hudson Bay	64	0.7249	0.0354	0.7004	0.0184	7.60	3.57	7.36	-0.003	0.034
James Bay	61	0.7009	0.0452	0.6871	0.0190	6.40	2.67	6.31	**0.050**	0.02
Sanikiluaq	253	0.7241	0.0367	0.7011	0.0092	9.90	4.01	7.39	-0.001	0.032
Global	580	0.7241	0.0371	0.7084	0.0060	10.80	4.08	7.41		

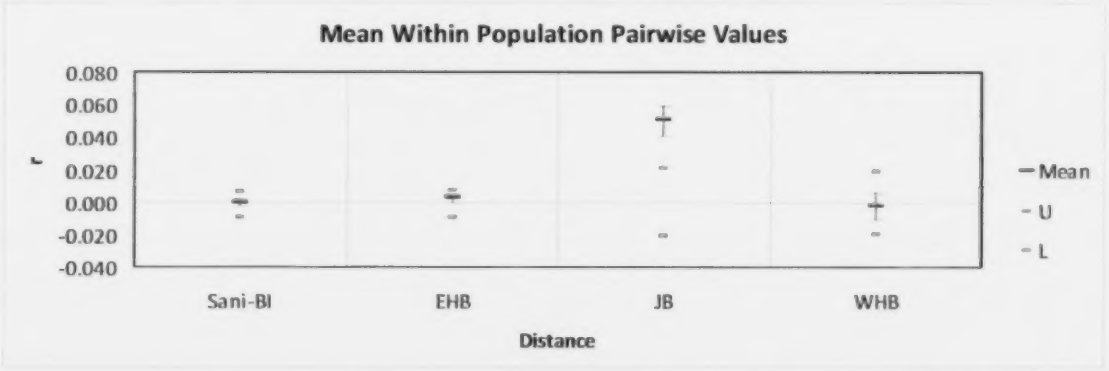

Figure 4. Mean within group relatedness of samples partitioned into main areas around James Bay. Confidence intervals (95%) obtained by permuting values around the null hypothesis of no differentiation from a relatedness of 0 (unrelated) are indicated with red bars. Confidence intervals (95%) around population means were obtained by bootstrap re-sampling.

*Table 6. Pairwise F_{ST} (frequency based measure of differentiation) for microsatellite differentiation among sample groups based on main area. Values below the diagonal are F_{ST} values, values above diagonal are the associated probabilities for differentiation. Values in **bold** indicate significant differentiation.*

	Eastern Hudson Bay	Western Hudson Bay	James Bay	Sanikiluaq
Eastern Hudson Bay	0	0.08333	**0.00833**	**0.00833**
Western Hudson Bay	0.0008	0	**0.00833**	**0.00833**
James Bay	**0.0153**	**0.0169**	0	**0.00833**
Sanikiluaq	**0.0028**	**0.004**	**0.0123**	0

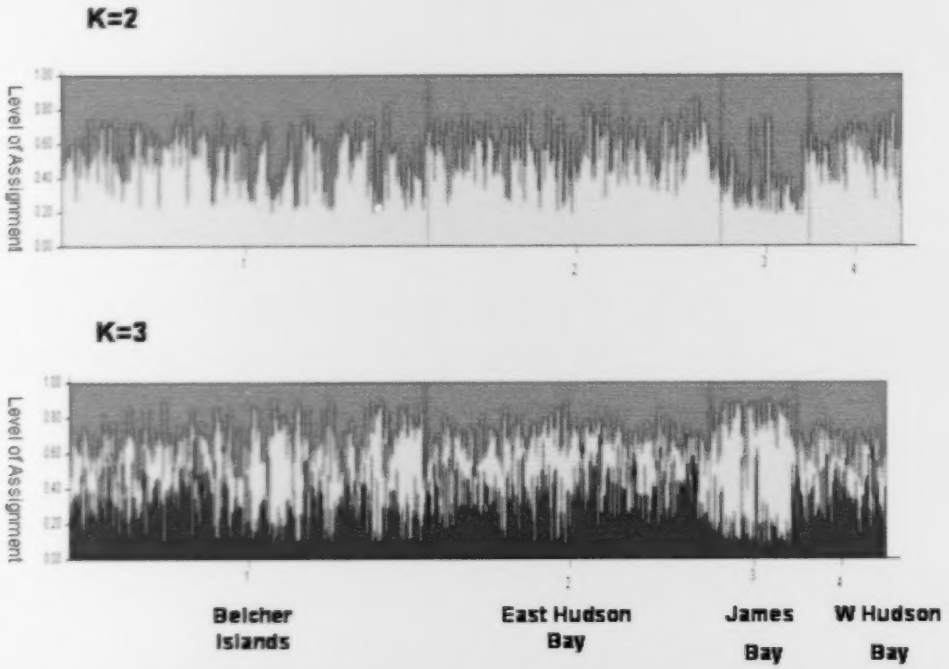

Figure 5. Results from individual based Bayesian analysis using the program STRUCTURE showing strength of assignment of individual samples to a pre-defined number of clusters (indicated by different colours on the bar graph). Results from K=2 clusters and K=3 clusters are shown with the samples grouped according to the four main areas used in this study. 1 = the Belcher Islands, 2 = Eastern Hudson Bay, 3 = James Bay, 4 = Western Hudson Bay

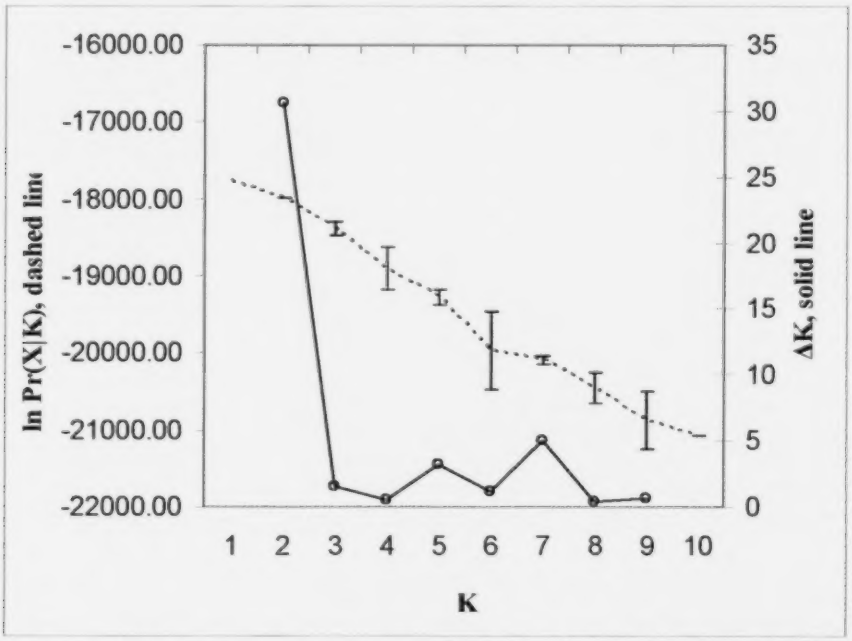

Figure 6. Results from individual based Bayesian analysis using the program STRUCTURE suggesting the presence of two genetic clusters for the beluga samples used in this study. Dashed line indicates log likelihood with error bars representing standard deviation calculated from three independent runs. Solid line represents K determined using the method of Evanno et al. (2005).

Inter-class PCR results (Figure 6a) echo the results of the STRUCTURE analysis. There is a certain degree of overlap in multilocus allelic compositions among all of the stocks, but there is also evidence that the James Bay samples form a more distant and distinct cluster. These results need to be evaluated in light of the high level of relatedness observed in the James Bay sample.

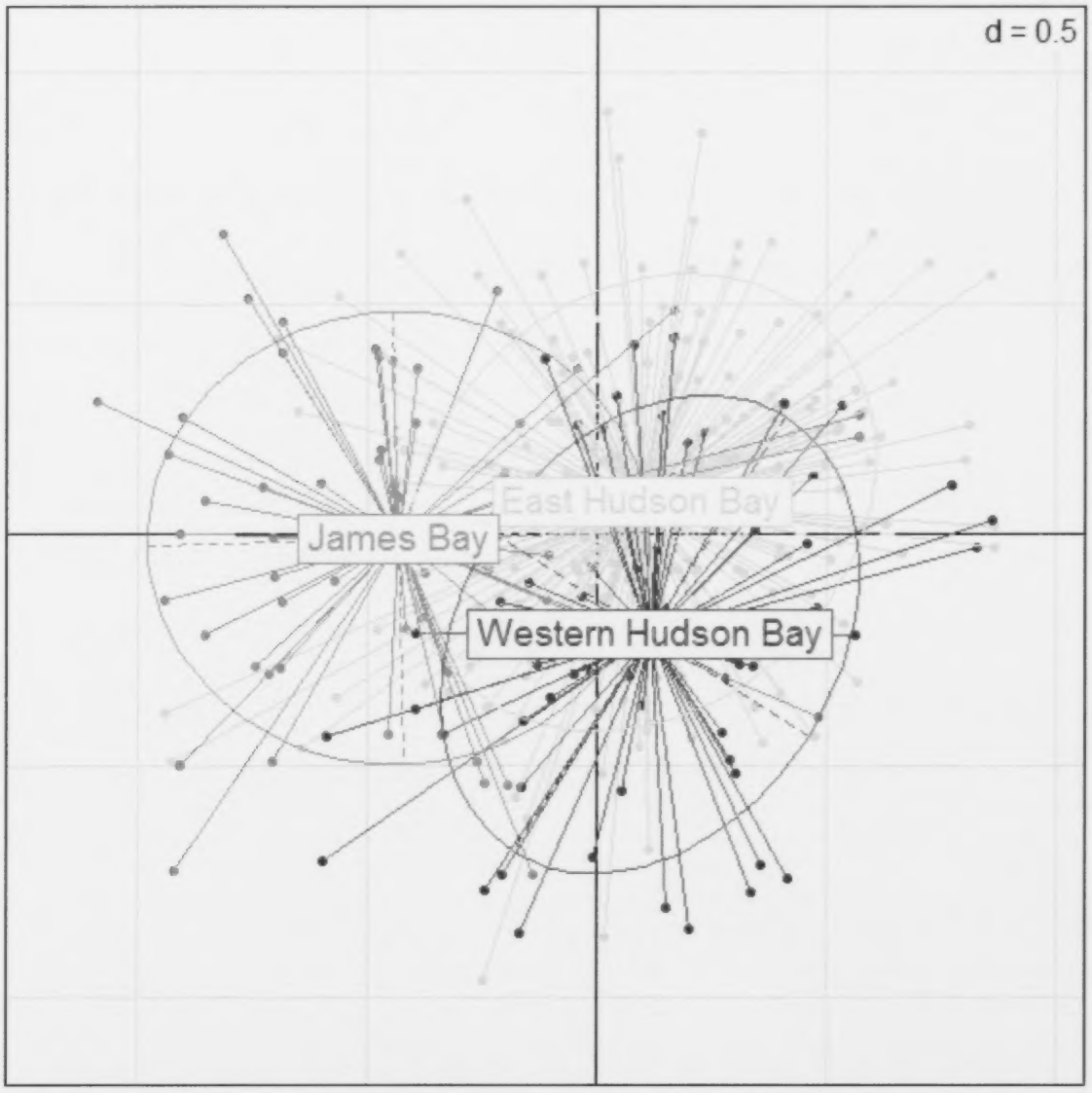

Figure 6a. Scatter plot of the first two axes of variation resulting from an interclass PCA conducted on belugas from James Bay, Western Hudson Bay and Eastern Hudson Bay. Individual samples are represented by dots connected to the centre of gravity for the sampling area of origin. The scale of both the x (58.5%) and the y (41.5%) axes are equal and the gridline dimensions are 0.5 units.

SATELLITE TELEMETRY

All 12 animals remained within JB throughout the deployment period (Fig. 7), which lasted on average 141 ± 40 days. Thus, they differed markedly in their seasonal movements from 17 EHB beluga equipped in Little Whale River, which had all migrated out of Hudson Bay by mid-November (Lewis et al. 2009, DFO unpubl. data). With the exception of three individuals that moved to the northern part of the bay in January (Fig. 8), beluga in James Bay generally did not venture far from their tagging site where they returned regularly until tag failure (5th November to 10th February).

With $x = 0$, the most likely value of the proportion of migrants was $\bar{p} = 0$, with a one-tailed 95% CI of 0 — 0.145 (Fig. 9a). The median value of $\bar{p}$ was 0.018 (Fig. 9b)

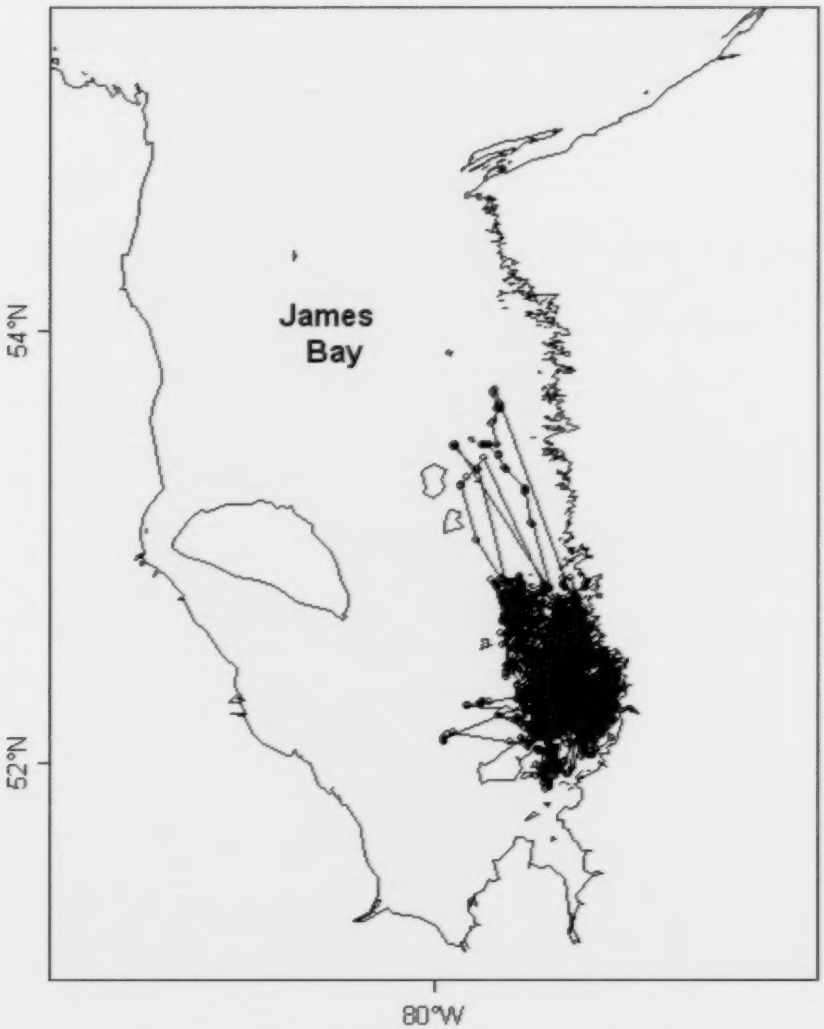

Figure 7. Relocations and movement tracks of 14 beluga whales equipped with satellite transmitters over the months August to February.

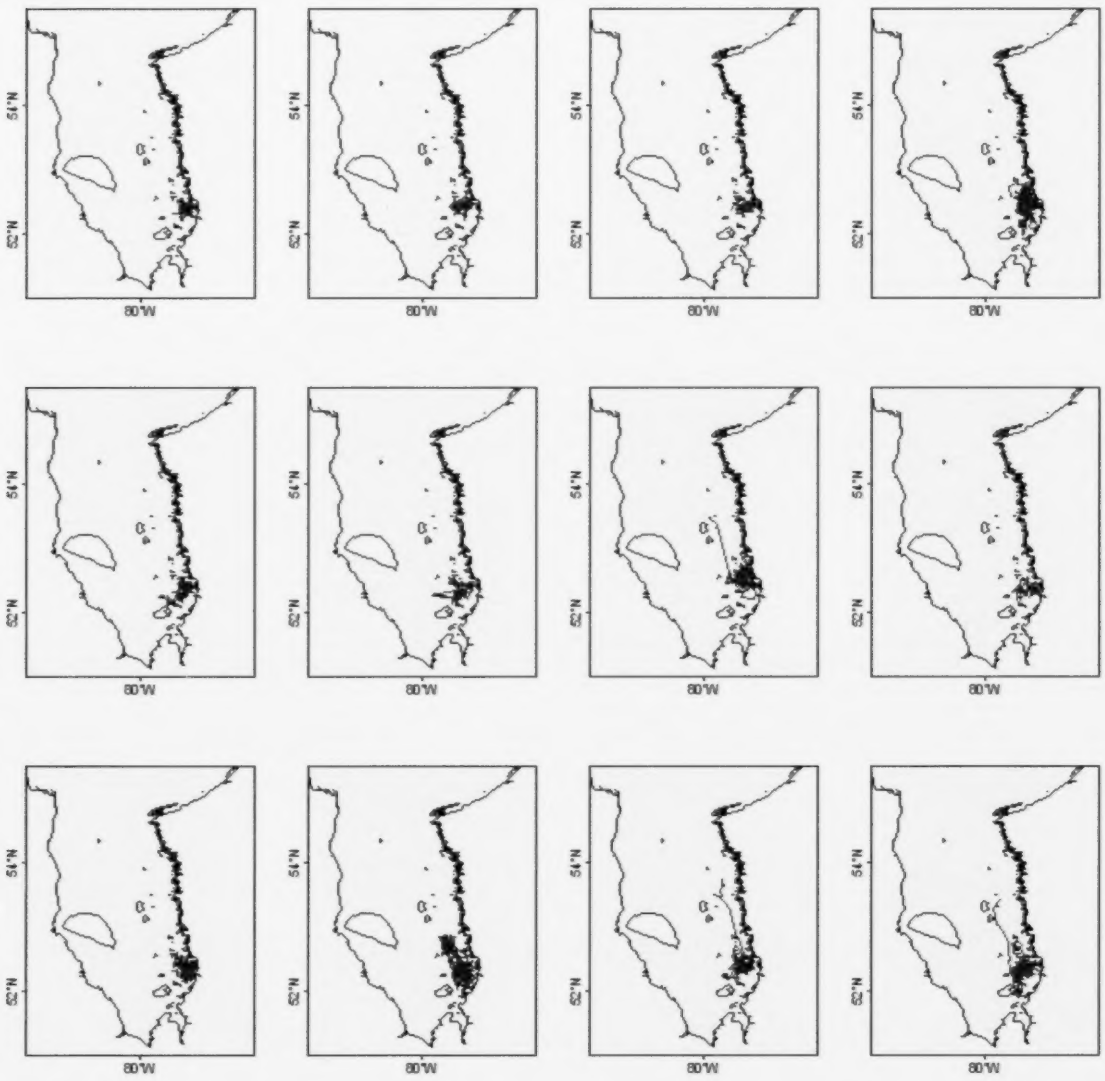

Figure 8. Relocations and movement tracks of the 12 beluga whales equipped with satellite transmitters for which deployment period lasted beyond the end of summer.

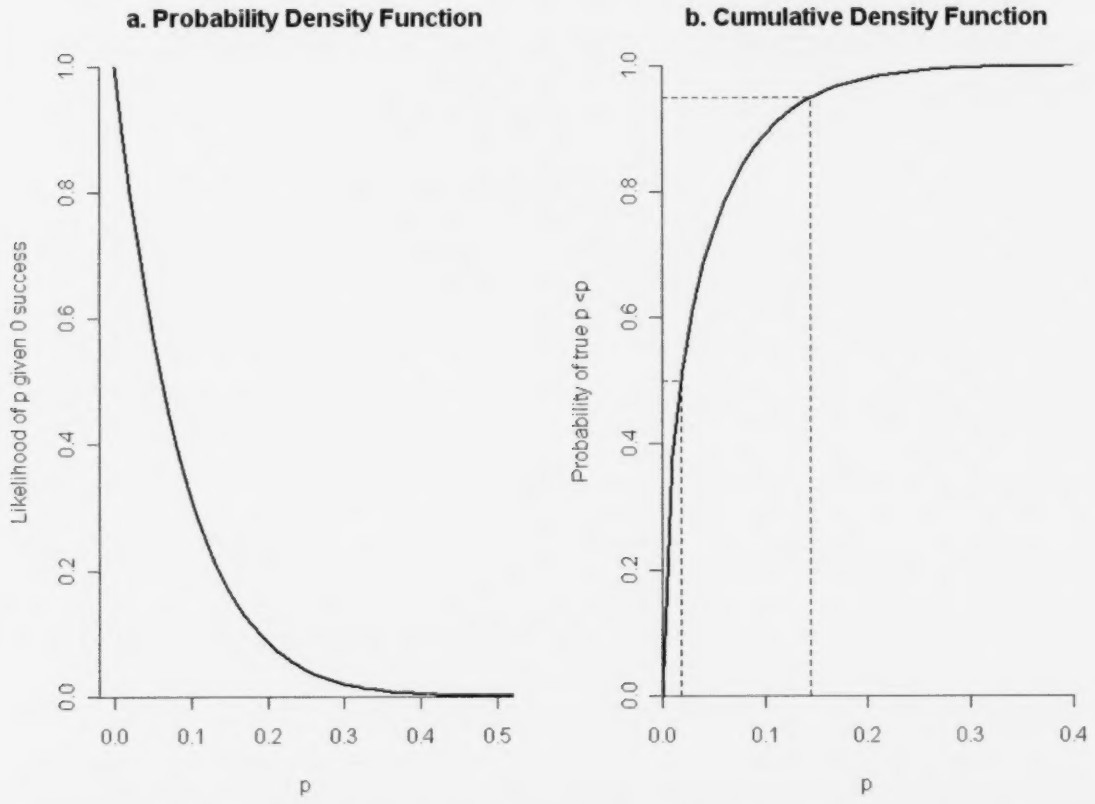

Figure 9. a.) Likelihood of values of the proportion p of migrants amongst James Bay beluga, given 0 migrants were observed out of 12 individuals equipped with satellite transmitters.
b.) Corresponding cumulative distribution showing the probability that the true value of p is equal to or lower than values on the x-axis. Dashed lines show the value of the median and the upper endpoint of the one-tailed 95% CI.

DISCUSSION

INFERENCE FROM GENETIC ANALYSES AND SATELLITE TELEMETRY

Of the 12 beluga whales equipped with satellite transmitters in James Bay, none showed migratory movement out of the bay during winter. Based on these observations, the most likely value of the proportion of migrants was 0%, but this value must be considered with caution. It is more conservative to state that there is a 95% probability that the true value of p is between 0 and 14.5%. The median estimate of p was 1.8%. In other words, it is likely that at least 98.2% of the James Bay beluga remain in the bay throughout the year.

This conclusion, however, is based on the assumption that the tagged individuals in our study are representative of the complete range of movements of James Bay beluga whales. We have no evidence that this is the case. Movement patterns and habitat use among tagged individuals were remarkably similar (Fig. 8), even in different years, but this may be due to the fact that they were all captured at the same site. Beluga are often observed in the estuary of Moose river, in the southern part of the bay, but this area was not visited by the individuals tagged at Cape

Hope Island. Moreover, aerial surveys have documented beluga in other parts of James Bay that do not overlap with the satellite tracks of our study (south-west, west and north-west of our observations, e.g., Smith and Hammill 1986, Kingsley 2000, Gosselin et al. 2009). These other beluga may have different movement patterns, which complicates making inference about the whole population in James Bay. This idea is supported by the interclass-PCA analysis of genetic data which also suggests some sub-structuring of belugas in a wider James Bay sample. The tagged animals all shared a single haplotype and were highly related to one another, suggesting a single lineage specific to a particular area of the bay. Further investigation with existing samples and with new, additional James Bay samples will be pursued.

Tag battery life was insufficient to document the spring period. Consequently we have no knowledge of distribution patterns between February and June. However, it is unlikely that James Bay beluga whales undertook long-range migratory movements to Hudson Strait at this time if not initiated earlier in the year given the extensive ice coverage during winter in Hudson Bay and western Hudson Strait.

Results of genetic analyses of beluga samples from James Bay and adjacent areas also indicate that there is a distinct stock of belugas occupying James Bay in the summer and that it is likely that these animals do not migrate out of Hudson Bay to interbreed with other beluga summer stocks. Given the low level of nuclear differentiation observed it is likely that this is a recent isolation and/or that there is some ongoing gene flow. Samples were found in both east Hudson Bay and Sanikiluaq that had genetic signatures more typical of James Bay animals (e.g., haplotype E11) could indicate that some of these animals are being hunted at the edge of their range by hunters from Sanikiluaq and south eastern Hudson Bay. Given the long life span and complexity of seasonal movements, further analyses of the genetic data with respect to sex, harvest or capture location, year and date of sampling will be required to fully understand this system. Monitoring efforts should be maintained and emphasis given to the importance of precise location and date information with the collection of samples.

OTHER SOURCES OF INFORMATION

Participants in a study of Inuit traditional knowledge have clearly indicated the presence of beluga in James Bay during winter (Lewis et al. 2009, fig. 3d). Jonkel (1969) reports presence of beluga whales in polynia during winter helicopter surveys of the area. These observations suggest that beluga have overwintered in James Bay for decades.

Satellite tracking of beluga from the Nelson River indicates that WHB beluga do not overwinter in James Bay, but that some individuals follow a migration route along the southern shore of Hudson Bay and may venture briefly into northern James Bay during the fall (P. Richard, pers. comm.). Smith and Hammill (1986) have observed large concentrations of beluga in the north-west corner of James Bay. It is possible some of these beluga correspond to such visitors from WHB.

CONCLUSIONS

All available evidence points to the conclusion that beluga whales from James Bay constitute a separate summer stock and potentially there are some animals that form a separate population with limited or no mixing with other stocks/populations. Moreover, belugas from this stock seem to differ from those of other summer aggregations in that they do not undertake long-range migratory movements out of the bay during winter. Apparently, the occurrence of polynia and suitable food resources make it possible for a large, distinct group of beluga to remain year-round in James Bay.

James Bay beluga should thus be considered a separate stock for surveys, population estimates and management of the EHB stock.

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